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CMP-N-acetylneuraminic acid synthetase of Escherichia coli: high level expression, purification and use in the enzymatic synthesis of CMP-N-acetylneuraminic acid and CMP-neuraminic acid derivatives.

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The gene encoding CMP-N-acetylneuraminic acid (CMP-NeuAc) synthetase (EC 2.7.7.43) in Escherichia coli serotype O7 K1 was isolated and overexpressed in E.coli W3110. Maximum expression of 8-10% of the soluble E.coli protein was achieved by placing the gene with an engineered 5'-terminus and Shine-Dalgarno sequence into a pKK223 vector derivative behind the tac promoter. The overexpressed synthetase was purified to greater than 95% homogeneity in a single step by chromatography on high titre Orange A Matrex dye resin. Enzyme purified by this method was used directly for the synthesis of CMP-NeuAc and derivatives. The enzymatic synthesis of CMP-NeuAc was carried out on a multigram scale using equimolar CTP and N-acetylneuraminic acid as substrates. The resultant CMP-NeuAc, isolated as its disodium salt by ethanol precipitation, was prepared in an overall yield of 94% and was judged to be greater than 95% pure by 1H NMR analysis. N-Carbomethoxyneuraminic acid and N-carbobenzyloxyneuraminic acid were also found to be substrates of the enzyme; 5-azidoneuraminic acid was not a substrate of the enzyme. N-Carbomethoxyneuraminic acid was coupled to CMP at a rate similar to that observed with NeuAc, whereas N-carbobenzyloxyneuraminic acid was coupled greater than 100-fold more slowly. The high level of expression achieved with the E.coli synthetase, together with the high degree of purity readily obtainable from crude cell extracts, make the recombinant bacterial enzyme the preferred catalyst for the enzymatic synthesis of CMP-N-acetylneuraminic acid.

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